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QUEST OF NOVEL GH20 *N*-ACETYL HEXOSAMINIDASE TRANSGLYCOSYLATING CATALYSTS: FUNCTIONAL SCREENING, DATA MINING AND SEMI-RATIONAL MUTAGENESIS

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Lack of access to certain types of oligosaccharides is a severe bottleneck for advances in glycosciences. The transglycosylation activity of retaining glycoside hydrolases (GH) has been used to provide oligosaccharides. The main drawbacks of those enzymes are the competing hydrolysis reaction and the fact that the products are also substrates, thus needing a kinetic control of the reaction. Several approaches have been developed to overcome these, including mechanism modifications (e.g. glycosynthases, chemical rescue), functional screening and data mining to find natural transglycosidases, directed evolution and targeted mutagenesis [1].

Here we focused on *N*-acetyl hexosaminidases from family GH20 that catalyse removal or addition of GlcNAc and GalNAc. Despite sharing a substrate-assisted mechanism with GH85, for which several glycosynthases have been created [2], no successful GH20 glycosynthase has been reported. Thus, we turned to discovery and characterization of new GH20s and performing a systematic mutagenesis study. Several new GH20s of bacterial origin were isolated and described by functional screening and data mining, including transglycosidases able to synthesize lacto-*N*-triose, a valuable oligosaccharide [3], as well as genuine hydrolases. Mutational analysis of all residues within the catalytic domain which were unchanged in >99% of 371 aligned GH20 sequences was pursued. Indeed, it has been shown that targeting conserved residues increases the likelihood of finding advantageous mutations [4,5]. Furthermore, it allows for transfer of successful mutations to other GH20s to find new efficient transglycosidases.

Notably, even though most conserved residues occur within the first and second shell of substrate interaction, 9 residues inside the $(\beta/\alpha)_8$ barrel pointing toward the active site are also conserved. To the best of our knowledge, such residues were not studied, although one of them mutated by directed evolution of a GH29 enzyme improved the transglycosylation yield and was transferable to other GH29 members [6]. Here transglycosylation yields of mutants in first shell, second shell and other residues within the $(\beta/\alpha)_8$ barrel will be compared for GH20.

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